SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Stool DNA-Based Colorectal Cancer Screening Test

Device Trade Name: Cologuard™

Device Procode: [**TO BE INSERTED BY FDA]**

Applicant's Name and Address: Exact Sciences Corporation
441 Charmany Drive
Madison, WI 53719

Premarket Approval Application (PMA) Number
[TO BE INSERTED BY FDA]

Date of Panel Recommendation
[TO BE INSERTED BY FDA]

Date of Notice of Approval of Application
[TO BE INSERTED BY FDA]

II. INDICATIONS FOR USE

Cologuard is intended for use as an adjunctive screening test for the detection of colorectal neoplasia associated DNA markers and for the presence of occult hemoglobin in human stool. A positive result may indicate the presence of colorectal cancer or pre-malignant colorectal neoplasia. *Cologuard* is not intended as a replacement for diagnostic colonoscopy. *Cologuard* is intended to be used in conjunction with colonoscopy and other test methods in accordance with recognized screening guidelines. A positive result in *Cologuard*, as with any screening test, should be followed by colonoscopy. *Cologuard* is intended for patients who are typical candidates for colorectal cancer screening, adults of either sex, 50 years or older, who are at average risk for colorectal cancer.

III. CONTRAINDICATIONS

Cologuard is not suitable for everyone. This test is indicated for men and women, age 50 years or older, who are at average risk for development of colorectal cancer. Patients should inform their doctor if they:

- Have a history of colorectal cancer, adenomas, or other related cancers.
- Have had a positive result from another colorectal cancer screening method within the last 6 months.
- Have been diagnosed with a high-risk condition for colorectal cancer. High risk conditions include but are not limited to inflammatory bowel disease (IBD), chronic ulcerative colitis (CUC), Crohn's disease, Familial adenomatous polyposis (FAP), or a family history of colorectal cancer.

- Have been diagnosed with a relevant hereditary cancer syndrome. Examples include Hereditary non-polyposis colorectal cancer syndrome (“HNPCC” or “Lynch Syndrome”), or others including but not limited to Peutz-Jeghers Syndrome, MYH-Associated Polyposis (MAP), Gardner’s Syndrome, Turcot’s (or Crail’s) Syndrome, Cowden’s Syndrome, Juvenile Polyposis, Cronkhite- Canada Syndrome, Neurofibromatosis and Familial Hyperplastic Polyposis.

IV. WARNINGS AND PRECAUTIONS

Warnings

- Patients should be advised of the caution listed in the *Cologuard* Patient Guide. Patients should NOT drink the preservative liquid.

Precautions

- Patients should not provide a sample for *Cologuard* if they have diarrhea or blood in their urine or stool from bleeding hemorrhoids, bleeding cuts or wounds on their hands, rectal bleeding, or menstruation.
- The risks related to using the *Cologuard* collection kit are low, with no serious adverse events reported among people in a clinical trial. Patients should be careful when opening and closing the lids to avoid the risk of hand strain.
- To ensure the integrity of the sample, the laboratory must begin processing patient specimens within 72 hours of collection. Patients should send stool samples to the laboratory according to the instructions stated in the *Cologuard* Patient Guide.

V. DEVICE DESCRIPTION

Cologuard is an *in vitro* diagnostic device designed to analyze patients’ stool for the presence of colorectal cancer (CRC) and pre-malignant colorectal neoplasia (“Advanced Adenoma” or “AA”) through detection of hemoglobin, multiple DNA methylation and mutational markers, and the total amount of human DNA. Specifically, *Cologuard* is designed to detect three (3) independent families of markers that exhibit an additive association with CRC and AA. The first DNA family targets epigenetic changes in the form of gene promoter region methylation. The second DNA family targets specific point mutations in *KRAS*. The third family of markers is non-DNA based and detects occult hemoglobin. The specific DNA markers that *Cologuard* targets are: *NDRG4* promoter region hypermethylation, *BMP3* promoter region hypermethylation, and seven (7) *KRAS* gene point mutations, Additionally, Beta-actin (“*ACTB”*) is a reference gene used for confirmation and quantitative estimation of the total amount of human DNA present in each sample.

Cologuard uses the following reagent components:

DNA Capture Reagents
CAP BDS, Capture Beads

DNA Preparation Reagents
DEN SLN, Denaturation Solution
BIS SLN, Bisulfite Conversion Solution
NEU SLN, Neutralization Solution
DES SLN, Desulphonation Solution (Concentrate)

BND BDS, Binding Beads
DNA and *QuARTS* Supplementary Lot Information Card

***QuARTS* Assay Reagents**
CAR SLN, Carrier Solution
ELU BFR, Elution Buffer
MIX A, Oligo Mix A, Methylation
MIX B, Oligo Mix B, Mutation
ENZ, Enzyme Mix
D CAL 1, DNA Calibrator 1, High Methylation
D CAL 2, DNA Calibrator 2, Low Methylation
D CAL 3, DNA Calibrator 3, High Mutation
D CAL 4, DNA Calibrator 4, Low Mutation

Hemoglobin Assay Reagents
Hb PLATE, Hemoglobin Assay Plate
SMP BFR, Sample Buffer
CONJ, Antibody Conjugate
SUBS, Substrate
STP SLN, Stop Solution
Hb CAL, Hemoglobin Assay Calibrator
Hemoglobin Assay Supplementary Lot Information Card

In addition, the following components are required for use of *Cologuard*.

(1) *Cologuard* Collection Kit containing the patient instructions, a protein sample tube with stool collection stick and buffer, a stool collection container, a foldable plastic bracket, a liquid preservative and a mailing container.

(2) *Cologuard* DNA Control Kit containing:

- DNA Control 1, High and DNA Control 2, Low with specific copy numbers of relevant methylated and non-methylated DNA.
- DNA Control 3, Negative with a specific copy number of non-methylated DNA

(3) *Cologuard* Hemoglobin Control Kit containing:

- Lyophilized Hemoglobin Control 1, High and Hemoglobin Control 2, Low derived from human whole blood and plasma containing specific concentrations of human hemoglobin.
- Lyophilized Hemoglobin Control 3, Negative derived from human whole blood and plasma with no human hemoglobin.

(4) Ancillary Materials and Bulk Assay Reagents

- STL BFR, Stool Buffer
- TABLT, Inhibitor Removal Tablet
- FILT, Spin Filter
- TUBES, Barcoded Mixing Tubes
- PRE WSH, Capture Bead Pre-wash
- CAP SLN, Capture Solution
- CAP WSH, Capture Wash
- BND SLN, Binding Solution

- CNV WSH, Conversion Wash Concentrate
- Hb WSH, Hemoglobin Assay Wash Concentrate

(5) BioTek ELx808 Absorbance Microplate Reader multichannel ELISA reader.

(6) Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with integrated thermal cycler and fluorimeter.

(7) Capture Incubator for automation of DNA capture hybridization.

(8) Capture Aspirator for automation of DNA capture clean-up washes.

(9) Hamilton Microlab®[1] STARlet for automation of the DNA preparation and *QuARTS* assay set up process.

(10) Exact Sciences System Software with Cologuard Test Definition.

(11) Other general lab equipment specified (centrifuge, shaker, bottle top dispenser, mixer etc.).

Principles of Operation

Cologuard involves stool DNA-based (sDNA) testing, which detects molecular markers of altered DNA that are contained in the cells shed by CRC or AA into the lumen of the large bowel. The DNA markers are released from cells that continuously slough from the lining of the colon into the stool. Through the use of selective enrichment and amplification techniques, sDNA tests are designed to detect even very small amounts of the DNA markers to identify CRC or AA. In addition, the test incorporates detection of fecal occult hemoglobin.

Stool samples are collected using the Cologuard Collection Kit, which includes patient instructions, a protein sample tube with stool collection stick and buffer, a stool collection container, a foldable plastic bracket, a liquid preservative, and a mailing container. The mailing container is used to send the collected sample to a lab for processing.

Once received, the stool sample is weighed, diluted, homogenized, and aliquots of the homogenates are taken and frozen. After pre-processing the *Cologuard* test begins with: (1) target specific capture to isolate DNA from frozen stool homogenates; (2) bisulfite conversion of methylated DNA; and (3) DNA purification coupled with Quantitative Allele-Specific Real-time Target and Signal (*QuARTS*™) amplification.[2] The *QuARTS* amplification technology combines the routinely used molecular biology techniques of real-time PCR and invasive cleavage chemistry to perform allele-specific amplification and detection of methylated target DNA (*NDRG4, BMP3)*, specific DNA point mutations (*KRAS*) and total human DNA (*ACTB)*. In a parallel workflow, a quantitative Enzyme-Linked Immunosorbent Assay (ELISA) technique is used to analyze the level of hemoglobin present in the stool sample. This panel of markers increases the likelihood of detection of CRC or AA, given the molecular heterogeneity of colorectal neoplasia. The final *Cologuard* result is determined utilizing a composite score based on a patient's individual methylation, mutation, and hemoglobin assay results. The score is calculated by multiplying a patient's individual methylation, mutation, and hemoglobin assay results by a constant marker specific weighting factor. The aggregate of these individually weighted

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1 Microlab® is a registered trademark of Hamilton Company.

2 *QuARTS*™ is a trademarked brand name that the company uses with the product.

marker results determines the composite score, which is then compared to a cut-off to determine a positive or negative result.

VI. ALTERNATE PRACTICES AND PROCEDURES

Conventional screening for CRC includes both invasive and non-invasive options. Invasive tools include flexible sigmoidoscopy, double contrast barium enema, computed tomography colonography (CTC) and conventional colonoscopy. Colonoscopy is considered to be the most accurate screening tool and is the reference method.

Other than stool DNA-based testing, non-invasive CRC screening tools include guaiac-based fecal occult blood testing (gFOBT) and immunochemical-based fecal occult blood testing (FIT).

Patients who have a positive test on an invasive or non-invasive screening, with the exception of colonscopy itself, warrant further investigation through conventional colonoscopy to rule out and/or remove the presence of CRC or AA.

VII. MARKETING HISTORY

Cologuard has not been marketed in the United States or any foreign country. *Cologuard* will be made available for sale in the United States.

VIII. POTENTIAL ADVERSE EFFECTS OF DEVICE ON HEALTH

Due to the nature of the noninvasive stool collection process, potential adverse events (AEs) caused by or related to testing with *Cologuard* are unlikely. During the pivotal clinical trial of 12,776 patients, only 4 adverse events were reported, none of which were believed to be associated with the test. The primary risk associated with the *Cologuard* test is a false assay result (i.e., a false positive or a false negative result). All positive test results should lead to a colonoscopy. Adverse events commonly associated with colonoscopy include abdominal discomfort and bowel irregularity post-procedure. Rare adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to the sedation resulting in respiratory and/or cardiac events, stroke and death. In the instance of a false negative result on *Cologuard*, there is a possibility that a case of CRC or AA could go undetected.

IX. SUMMARY OF ANALYTICAL STUDIES

Nonclinical studies were conducted by Exact Sciences to evaluate the analytical performance characteristics of *Cologuard*. The studies are described below.

A. Algorithm Development and Cut-Off Determination.

The cut-offs and the algorithm for the *Cologuard* sDNA-based colorectal cancer screening test were established based on an evaluation of a panel of donor samples that were categorized by colonoscopy. Variable selection for the *Cologuard* model was performed as a stepwise selection with the main variables assessed one at a time based on their respective statistical significance. The total sample size of the dataset for algorithm development included 953 samples, including 794 normal pathology samples, 73 advanced adenomas and 86 cancers.

The derived *Cologuard* algorithm sensitivity and specificity compared to colonoscopy outcome was assessed based on a data set of 1003 samples that included the original 953 samples used to build the algorithm, plus 50 samples tested with the hemoglobin component of *Cologuard,* but collected with a different protein collection tube. The achieved sensitivity of approximately 98% for cancer and approximately 57% for advanced adenoma met the acceptance criteria.

After the initial cut-off was determined for *Cologuard*, the company verified the robustness of the logistic regression-based predictive algorithm and refined the risk score cut-off using a combination of computer simulations and statistical cross-validation techniques such as Leave-One-Out cross-validation ("LOOCV") and 10-fold cross-validation analyses. Furthermore, various simulations were also performed on the *Cologuard* cut-off study data (n=953) to determine the best estimate of *Cologuard* precision.

B. Sensitivity: Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantification (LoQ) and Linearity.

LoB, LoD, and LoQ studies were performed for both the methylation and mutation component (i.e., molecular assay) and the hemoglobin assay component of *Cologuard* based on guidance from the CLSI Standard: EP17-A (*Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline*). For molecular assays, such as the *QuARTS* component of *Cologuard*, the signal from the blank wells is absent. Therefore, the LoD and LoQ were established through means independent of a Limit of Blank (LoB) measurement.

Linearity and Linear Range studies using concentrations above and below the anticipated linear range were tested in the molecular assay and hemoglobin assay components of *Cologuard*. Linearity studies were performed based on guidance from CLSI Standard: EP6-A (*Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline).*

Analytical sensitivity characteristics for *Cologuard* were observed as follows:

Table 1: Analytical Sensitivity Characteristics Summary

Performance Characteristic	Molecular Assay	Hemoglobin Assay
Limit of Blank	Not Applicable	0.4 ng/mL
Limit of Detection	Methylation Markers: *NDRG4, BMP3* and *ACTB* 0.702 to 0.738 log strands Mutation Markers: *KRAS* 1.058 log strands	1.3 ng/mL
Limit of Quantification	LoQ ≤ 1.176 log strands	4.8 ng/mL
Assay linearity	$R^2 = \geq 0.996$ Linear range = 1.1760 to 5.591 log strands	Linear range = 4.8 ng/mL to 500 ng/mL No hook effect observed for concentrations up to 100 µg/mL

C. *Cologuard* Molecular Assay Cross-Reactivity with Wild Type KRAS.

Exact Sciences evaluated the potential for cross-reactivity with wild type *KRAS* by testing two levels of *KRAS* wild type DNA in the *Cologuard QuARTS* methylation and mutation assays. *KRAS* wild type DNA was assessed at levels of 20,000 copies of wild type *KRAS*, which is greater than the average expected to be seen in normal human stool samples, and 200,000 copies of wild type *KRAS*, 10 times higher. Average strand recovery and standard deviations for *NDRG4, BMP3, KRAS1,* and *KRAS2* were calculated. The percentage of cross-reactivity of the two levels of wild type *KRAS* for the *QuARTS* Mutation and methylation assays was determined, and cross-reactivity percentages for each of the test levels and no target control ("NTC") were calculated after subtracting the background NTC.

Results from this study indicated that cross-reactivity for wild type *KRAS* at 200,000 copies was 0% for the methylation assay and 0.01% for the mutation assay. These results are highlighted in **Table 2** below.

Table 2: Percent of Cross-Reactivity to Wild Type *KRAS*, by Assay

	Methylation Assay*		
	NDRG4	***BMP3***	***BTACT***[1]
Wild Type *KRAS* Level	**Mean Strands**	**Mean Strands**	**Mean Strands**
400,000 Strands (200,000 Copies)	0%	0%	0%
40,000 Strands (20,000 Copies)	0%	0%	0%
	Mutation Assay*		
	KRAS1	***KRAS2***	***ACT***[2]
Wild Type *KRAS* Level	**Mean Strands**	**Mean Strands**	**Mean Strands**
400,000 Strands (200,000 Copies)	0.01%	0.01%	0%
40,000 Strands (20,000 Copies)	0%	0%	0%

*When strand levels derived from the cross-reactivity reactions were below the LOD of the respective reaction, a cross-reactivity level of 0% was assigned.
[1] *BTACT* refers to how the *Cologuard* software characterizes the *ACTB* in the methylation assay.
[2] *ACT* refers to how the *Cologuard* software characterizes the *ACTB* in the mutation assay.

D. *Cologuard QuARTS* Partial Methylation Testing.

Many genes have elevated methylation in their promoter region in CRC, whereas the same genes have low levels of methylation in normal colon epithelial cells. Exact Sciences previously demonstrated that highly methylated promoter region sequences in BMP3 and NDRG4 correlates to CRC and AA and low level methylation correlates to normal tissue with the *QuARTS* technology.

The DNA oligonucleotides used in the *Cologuard* methylation assay are designed to be a perfect match to fully methylated DNA in *NDGR4* and *BMP3*.

The company conducted testing to demonstrate that the assay was specific for highly methylated DNA. The analytical specificity of the DNA methylation assay component of *Cologuard* was tested against partially methylated *BMP3* and *NDRG4* DNA targets using the *QuARTS* assay. The testing utilized synthetic DNA targets that contained all possible permutations of partial methylations in the *QuARTS* assay footprint region of *BMP3* and *NDRG4*.

The study results demonstrated that *Cologuard* is specific for highly methylated DNA, specifically highly methylated *NDRG4* and *BMP3*. At least five sites of eight for *BMP3* and five sites of nine for *NDRG4* have to be methylated for any reactivity in *Cologuard*. With respect to *NDRG4*, the percent cross-reactivity was 2.5%, indicating that the analytical specificity for total methylations in *NDRG4* is 97.5%. With respect to *BMP3*, the percent cross-reactivity was 1.8%, indicating that the analytical specificity for total methylations in *BMP3* is 98.2%, above the 95% specificity outlined in the acceptance criteria.

E. *Cologuard* Hemoglobin Assay Cross-Reactivity and Specificity.

The ability of the Hemoglobin Assay to detect hemoglobin in specimens heterozygous for Hemoglobin S (HbS) and Hemoglobin C (HbC) was evaluated. Samples used for testing Hb variants consisted of a stool sample background spiked with normal, HbS heterozygous, or HbC heterozygous whole blood. The Hemoglobin Assay detected both HbS and HbC variants, when comparing equivalent volumes of blood from normal and heterozygous variant specimens.

Additionally, cross-reactivity of *Cologuard* Hemoglobin Assay with animal hemoglobin and myoglobin was evaluated. Samples used for testing animal blood cross-reactivity consisted of a stool sample background spiked with animal whole blood. Samples used for testing myoglobin cross-reactivity consisted of a stool sample background spiked with prepared meat extracts or purified myoglobin. Thirteen replicates of each sample type were tested with the *Cologuard* Hemoglobin Assay.

Mean HbC concentrations for all animal hemoglobin and myoglobin samples were less than the limit of detection (LoD) of the assay (1.3 ng/mL) after the mean concentration of the Hb Negative Stool Sample was subtracted, indicating that no cross-reactivity was detected.

F. *Cologuard* Cross-Reactivity with Non-Colorectal Cancers and Diseases.

Exact Sciences evaluated the potential for cross-reactivity with non-colorectal cancers by testing 151 specimens from subjects with other cancers, including diseases other than CRC that have a potential association with the GI tract, or inflammatory conditions that could affect the screening population for *Cologuard*. The diseases and cancers tested are listed in **Table 3** below. Samples were tested with both the molecular and hemoglobin assay components of *Cologuard*. Overall *Cologuard* Scores were then generated to assess whether reactivity was found with any of these non-CRC samples.

Cancers in organs connected to the digestive tract (i.e., pancreas and liver) may shed markers that could be detected by *Cologuard.* As such, it is expected that a certain level of reactivity will be observed in cases of these cancers. The results are highlighted in **Table 3** below.

Table 3: Incident Rates and Contribution to *Cologuard* Positivity for Non-CRC Diseases and Cancers

Disease or Cancer*	Number of specimens	Incident rate per	% Positivity of *Cologuard*	Number additional positive *Cologuard*

	tested	10,000**		call in 10,000 subjects
Bladder Cancer	17	2.3	- -	- -
Breast Cancer	14	12.4	- -	- -
Esophagus Cancer	11	0.5	- -	- -
Gynecologic Cancer	11	2.0	36.4%	0.7
Hepatic Cancer	6	0.8	50%	0.4
IBD	18	1.0	38.9%	0.4
Lung Cancer	10	6.5	- -	- -
Lupus	17	0.2-0.8	- -	- -
Pancreas Cancer	12	1.2	41.6%	0.5
Prostate Cancer	12	15.5	- -	- -
Rheumatoid Arthritis	15	4.1	- -	- -
Stomach Cancer	8	0.8	- -	- -
Total per 10,000 subject		NA	NA	2.0

*Listed value for gynecologic cancer is the sum of ovarian and cervix uteri cancers.
**For cancers, figures were obtained from the National Cancer Institute (http://seer.cancer.gov/statfacts/index.html). For other diseases, figures were obtained from the Centers for Disease Control and Prevention (http://www.cdc.gov).

Based on the results of this study, the expected positivity for the tested diseases would result in only a minimal (0.02%) decrease in specificity for *Cologuard* (or two positive calls per 10,000 screening patients tested).

G. Precision and Reproducibility (Lab-to-Lab).

A laboratory-to-laboratory precision and reproducibility study was performed to assess variation of the *Cologuard* assay measurement system based on guidance from the CLSI Standard: EP15-A2 (*User Verification of Performance for Precision and Trueness; Approved Guideline*). As part of the study, a variance component analysis was performed by sample type for the *Cologuard* system to estimate the components of precision for each source of variation (operator, run, site, and replicate) as well as total variation for each individual marker and the overall *Cologuard* Score.

The study was performed at three sites (100, 200, 300), with a minimum of two operators at each site. A total of 22 *Cologuard* runs were performed at each site, 11 per operator. Each run involved 42 samples, including six replicates of each of the following: four stool pool samples (negative, high negative, low positive and high positive) and three control samples (negative, low positive and high positive), supplied by Exact Sciences.

For the molecular assay component of *Cologuard*, the stool sample types were prepared by combining characterized residual stool samples available to Exact Sciences. The samples were characterized as positive or negative for CRC based on colonoscopy results. Subsequently, these residual clinical stool specimens were tested with the *Cologuard* assay to establish the planned DNA content of samples for use in this study. Spiked synthetic DNA was used to create the contrived control samples.

For the hemoglobin assay component of *Cologuard*, the clinical stool pools were prepared by adding fresh whole blood to normal patient stool pools. Specifically, whole blood was spiked into stool samples and diluted to the appropriate concentration. Control samples (including negative, low, and high controls) were provided to each testing site in lyophilized form for reconstitution prior to testing.

Percent agreement between sites was evaluated by generating two-by-two (2 x 2) contingency tables for negative and positive results for all site pairs, calculating the average positive agreement (APA) and average negative agreement (ANA), and calculating the exact two-sided lower 95%

confidence interval by the Clopper-Pearson method. The resulting lower confidence limit was then compared to the target agreement rate of 0.95. The lower confidence interval for percent agreement of all site pairs was ≥0.95. Inter-site agreement is shown in **Table 4** and shows minimal variation.

Table 4: Inter-site Agreement

Site Comparison	Number Agreed	Total Compared	Agreement Rate	95% CI Lower Bound***
ANA* – Site 100 and Site 200	768	777	0.988	0.978
APA** – Site 100 and Site 200	1026	1035	0.991	0.983
Site Agree – Site 100 and Site 200	897	906	0.990	0.982
ANA – Site 100 and Site 300	744	746	0.997	0.990
APA – Site 100 and Site 300	1012	1014	0.998	0.993
Site Agree – Site 100 and Site 300	878	880	0.998	0.992
ANA – Site 200 and Site 300	756	764	0.990	0.979
APA – Site 200 and Site 300	1004	1012	0.992	0.984
Site Agree – Site 200 and Site 300	880	888	0.991	0.982

*ANA = Average negative agreement
**APA = Average positive agreement
***Clopper-Pearson Confidence Interval

Descriptive statistics were separately calculated for all marker/sample combinations. %CV was calculated only for samples with an expected positive result. Inter-site descriptive statistics are provided below (**Table 5**).

Table 5: Inter-Site Descriptive Statistics for the *Cologuard* Score

Sample	Variable	N	Mean	Lower 95% CL for Mean	Upper 95% CL for Mean	Std Dev	Total %CV
Negative Stool Pool	*Cologuard* Score	387	9.98	9.65	10.31	3.31	NA
High Negative Stool Pool		394	62.92	60.24	65.61	27.14	NA
Low Positive Stool Pool		393	391.11	383.66	398.36	74.13	18.96
High Positive Stool Pool		394	978.34	977.44	979.24	9.13	0.93
Negative Control		392	6.35	6.26	6.44	0.90	NA
Low Positive Control		393	626.24	621.39	631.09	48.91	7.81
High Positive Control		393	963.38	962.30	964.46	10.89	1.13

Overall the assay was highly reproducible with inter-site agreement values of the lower confidence interval of >95% (Table 4) and all of the positive *Cologuard* Scores had inter-site CVs of less than 20% (**Table 5**).

H. Lot-to-Lot Reproducibility.

Lot-to-Lot reproducibility was evaluated for *Cologuard* based on guidance from the CLSI Standards: EP5-A2 (*Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*); EP15-A2 (*User Verification of Performance for Precision and Trueness; Approved Guideline*); EP12-A2 (*User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline*); and I/LA28-A2 (*Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays; Approved Guideline*).

Lot-to-Lot reproducibility was assessed by testing a sample panel comprised of seven samples containing various levels of DNA and hemoglobin, using three lots of *Cologuard* reagents and controls.

For the molecular assay component of *Cologuard*, the stool sample types were prepared by combining characterized residual stool samples available to Exact Sciences. The samples were characterized as positive or negative for CRC based on colonoscopy results. Subsequently, these residual clinical stool specimens were tested with the *Cologuard* assay to establish the planned DNA content of samples for use in this study. Spiked synthetic DNA was used to create the contrived control samples.

For each sample in the panel, there were 24 sample results per lot and 72 sample results for the entire study. Across the seven samples in the panel, there were 168 results per lot, and 504 results for the entire study.

The mean, SD, %CV, N, minimum value and maximum value were calculated for each marker or each lot and test sample. Additionally, *Cologuard* Scores were determined. Percent positive results for the *Cologuard* Score were analyzed across lots and for lot to lot. Variance component analyses were also conducted.

Descriptive statistics were calculated for all marker/sample combinations, including median, mean, mean upper and lower 95% confidence intervals, standard deviation, and coefficient of variation values. %CV was calculated only for controls with expected result of positive. Descriptive statistics were calculated both within and across lots. Descriptive statistics for this study are shown below. The *Cologuard* Score %CV value for positive samples were within the pre-specified acceptance criteria, ranging between 0% and 16.8%.

Table 6: Descriptive Statistics for Lot-to-Lot *Cologuard* Score

Sample Name	N	Median	Mean	Lower 95% CL for Mean	Upper 95% CL for Mean	Std Dev	CV
Negative Stool Pool	72	9.47	11.39	10.19	12.58	5.07	NA
High Negative Stool Pool	72	64.46	57.74	51.12	64.36	28.18	NA
Low Positive Stool Pool	71	380.75	373.93	359.03	388.84	62.98	16.84
High Positive Stool Pool	71	973.92	972.88	970.36	975.40	10.64	1.09
Negative Control	70	6.33	6.40	6.21	6.59	0.79	NA
Low Positive Control	71	584.09	579.52	570.09	588.95	39.85	6.88
High Positive Control	71	1000	1000	1000	1000	0	0

Percent agreement between lots was evaluated by generating 2 x 2 tables for negative and positive results for all lot pairs, calculating the average positive agreement (APA) and average negative agreement (ANA). Testing of samples with various levels of hemoglobin and DNA markers demonstrated a percent agreement for positive and negative samples across multiple lots between 98.6% and 100%, with a lower confidence limit above 95%.

Table 7: **Lot-to-Lot Percent Agreement**

Lot Comparison	Number Agreed	Total Compared	Agreement Rate	95% CI Lower Bound***
ANA* - Lot1 and Lot2	142	142	1.0000	0.9744
APA** - Lot1 and Lot2	188	188	1.0000	0.9806
Lot Agree - Lot1 and Lot2	165	165	1.0000	0.9779
ANA - Lot1 and Lot3	140	142	0.9859	0.9501
APA - Lot1 and Lot3	180	182	0.9890	0.9609
Lot Agree - Lot1 and Lot3	160	162	0.9877	0.9561
ANA - Lot2 and Lot3	142	144	0.9861	0.9507
APA - Lot2 and Lot3	184	186	0.9893	0.9617
Lot Agree - Lot2 and Lot3	163	165	0.9879	0.9569

NOTE: Proportion values are point estimates used to determine the Clopper-Pearson 2-sided Confidence Interval. Only Clopper-Pearson Lower Limit values are shown in the above table.

*ANA = Average negative agreement
**APA = Average positive agreement
***Clopper-Pearson Confidence Interval

The study demonstrated that *Cologuard* results are reproducible across multiple reagent lots.

I. Robustness

Exact Sciences assessed the *Cologuard* performance in response to defined variable factors (see below) at specific steps in the test procedure, using both the molecular assay and hemoglobin assay components of *Cologuard*. The processing steps analyzed in this study are the steps at which operator variability or error are most likely to occur. Three total instrument and operator sets were used for the study

For the molecular assay component of *Cologuard*, results when these various factors were introduced into the processing steps were compared to the expected results for a positive stool sample, a control sample with high levels of mutation and methylation markers, and a control sample with moderate levels of mutation and methylation markers. Fourteen replicates of each sample type were used. Analysis of these samples assumed a hemoglobin value of zero, when calculating overall *Cologuard* score. Factors tested included the following:

- Factors related to DNA capture, including wait times between processing steps, amount of reagents added, and duration of storage at the appropriate temperatures;
- Factors related to the amount of time various instruments are paused during the automated DNA preparation and *QuARTS* assay steps of the *Cologuard* process; and
- Factors related to the amount of time between plate assembly and processing during the *QuARTS* assay step.

For the hemoglobin assay component of *Cologuard*, results when these factors were introduced into the processing steps were compared to the expected results for a stool sample with a known level of endogenous hemoglobin and a high and low control sample with high and low levels of hemoglobin. The study tested 16 replicates of each sample type. Analysis of these results involved comparing the resulting hemoglobin concentration with the expected hemoglobin concentration. Factors tested include the following:

- Time between steps during plate preparation;
- Incubation times for antibodies and substrates; and
- Time between steps during plate reading phase.

The results for the molecular assay component of *Cologuard* showed that time between plate assembly and processing during the *QuARTS* assay step and the number of days the captured DNA was stored at the appropriate temperatures could have a detectable effect on assay response. Testing demonstrated that the prepared *QuARTS* plate should be processed within 30 minutes and captured DNA could be stored for up to four days.

Results for the hemoglobin assay component of *Cologuard* showed that substrate incubation time had a detectable effect on assay performance. Testing demonstrated that a substrate incubation time of 15 ± 1.5 minutes would result in acceptable assay performance.

J. Interfering Substances

Cologuard Molecular Assay Interference Testing.

Interference with the molecular assay component of *Cologuard* was evaluated using 55 common substances that potentially could be present in stool materials. Testing was performed using 16 replicates of positive and negative stool homogenate samples, with and without interfering substances. All samples were processed through the entire molecular test component of *Cologuard*, evaluating the methylation and mutation markers for *Cologuard* score calculations to assess whether interference was observed.

Cologuard molecular assay was evaluated with potential interfering substances in the following categories:

- Common lotions, creams, and feminine over-the-counter products;
- Stool softeners, anti-diarrhea, and laxative products;
- Anti-acids and upset stomach relief products;
- Animal genomic DNA of commonly edible animals (both high and low levels);
- Urine and alcohol;
- A mixture of common vegetables and fruits; and
- Fecal Fats (fatty acids and cholesterol).

For samples known to be positive, no differences were observed in the overall *Cologuard* results for spiked samples versus unspiked samples. Comparisons of the mean *Cologuard* score for each interferent group with the mean score for the unspiked control revealed no statistically significant differences. No interference with the molecular assay component of *Cologuard* was observed for any of the tested substances.

Cologuard Hemoglobin Assay Interference Testing.

Interference with the hemoglobin assay component of *Cologuard* was evaluated using 46 common substances that potentially could be present in stool materials. Testing was performed using 16 replicates of positive and negative stool homogenate samples, with and without interfering substances. All samples were processed through the hemoglobin assay component of *Cologuard.* Samples were evaluated for inhibition or enhancement of hemoglobin concentrations in spiked and un-spiked samples to assess whether interference was observed.

Cologuard hemoglobin assay was evaluated with potential interfering substances in the following categories:

- Common lotions, creams, and feminine over-the-counter products;
- Urine;
- Stool softeners, anti-diarrhea, and laxative products;
- Anti-acids and upset stomach relief products;
- Antibiotics, anti-inflammatories, anti-fungal drugs, pain relievers, and decongestants;
- A mixture of common vegetables and fruits;
- Fats and lipids; and
- Alcohol.

A comparison of the mean hemoglobin concentration results indicated there were no statistical differences between the mean hemoglobin concentrations in test and control samples in both the ‘positive’ and ‘normal’ stool pools. None of the substances tested interfered with the *Cologuard* hemoglobin assay.

K. Carry-over and Cross-contamination *Cologuard* Testing.

Carry-over Evaluation

Sequential runs of high positive and negative samples were used to evaluate carry-over contamination for each assay component of *Cologuard*. Testing of the molecular assay and hemoglobin assay components was conducted in two separate studies.

For the molecular assay (methylation/mutation assay), the testing involved two consecutive runs of high positive DNA samples, composed of 10x high level run controls diluted in Tris, EDTA and non-human DNA, followed by a run of negative samples composed of Tris, EDTA and non-human DNA. A total of 43 high positive samples and 3 run controls were used in each high positive run. A total of 43 negative samples and 3 run controls were used for the negative run.

For the hemoglobin assay, the testing involved two consecutive runs of high positive hemoglobin samples, composed of 100,000 ng/mL hemoglobin, followed by a run of negative samples composed solely of the protein preservative solution from the hemoglobin sample collection tube. The high positive samples consisted of a hemoglobin level that is much higher than the quantitative range of the assay, which identifies all samples >500 ng/mL as greater than the maximum range of the assay. For the high positive runs, a total of 86 high positive hemoglobin samples were used. For the negative run, 86 negative samples were used. In each run, the signal obtained on the controls was utilized to ensure the validity of the run.

Results from the molecular assay and hemoglobin assay carry-over analyses demonstrated that the *Cologuard* assay components and the instruments required for running the assay performed as expected and satisfied the acceptance criteria for the study.

Cross-contamination Evaluation

Cross-contamination testing of *Cologuard* was based on a checkerboard study design, alternating high positive and negative samples, to evaluate the potential for contamination from the positive to the negative samples within a run. Testing of the molecular assay and hemoglobin assay components was conducted in two separate studies.

For the molecular assay, 22 high positive samples, 21 negative samples, and three run control samples were used. As in the carry-over study, the high positive samples for this study were also composed of 10x high level run controls diluted in Tris, EDTA and non-human DNA, and the negative samples were composed of Tris, EDTA and non-human DNA. One run was performed and samples were processed using the *Cologuard* molecular process from the semi-automated front end sample processing through the automated processing.

For the hemoglobin assay, a total of 43 high hemoglobin and 43 negative hemoglobin samples were used. As in the carry-over study, the high positive samples contained 100,000 ng/mL hemoglobin, while the negative samples consisted solely of the protein preservative solution from the hemoglobin sample collection tube. Three runs were performed and samples were processed using the Cologuard hemoglobin process.

Results from the cross-contamination analysis for the molecular assay demonstrated that the molecular assay component of *Cologuard* and the associated instruments needed to run the assay performed as intended and met the study acceptance criteria. Specifically, one well experienced some cross-contamination (52 strands of ACTB), however, this was within the pre-specified acceptance criteria, which dictated that no more than three wells could exhibit 10-100 strands of ACTB and no single well could exhibit more than 100 strands.

The high hemoglobin samples utilized in this study contained hemoglobin levels that are approximately 50 times higher than the median positive hemoglobin values observed in colorectal cancer subjects (Levi et. al, 2007). The high hemoglobin concentrations tested in this study are much higher than would be expected in use of *Cologuard*. First run results showed a signal in 4 out of 43 negative samples with an average detectable hemoglobin level of 11 ng/mL (0.011%). As the hemoglobin assay involves several manual steps (e.g., manual washing and reagent addition), repeat testing was conducted, in which no cross contamination was observed. This result indicates that there is no cross-contamination from the automated equipment, but rather operator-induced cross-contamination can occur if procedures are not carefully followed. Data from the combined runs passed the pre-specified acceptance criteria described in the protocol.

L. Stability Studies.

In-Use Stability: Molecular Assay Stability Under Standard Operating Conditions.

The stability of reagents used in the molecular assay component of *Cologuard* was evaluated following guidance from CLSI standard: EP25-A (*Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*). The purpose of this testing was to determine reagent stability after opening the containers and using them under potential user operating conditions. All reagents required for the molecular assay were tested.

Samples were processed with the molecular assay component of *Cologuard*, using these reagents, to determine the in-use stability of the reagents and the effect of the various factors above on *Cologuard* results. The samples used in the in-use stability study for the various *Cologuard* reagent groups included DNA calibrators; High Positive and Low Positive control samples consisting of synthetic targets in stool collection buffer; a Negative DNA control sample; DNA positive and negative run controls; and a positive stool sample.

The study demonstrated that *Cologuard* reagents are stable when opened or stored for variable times before use under standard operating conditions. Specifically:

- Multiple-use reagents stored at room temperature are stable for up to six weeks from the open date.
- Capture Beads that have been pre-washed and stored at 2-8°C are stable for up to 13 days.
- Pre-washed Capture Beads are stable for up to six hours at room temperature prior to use.

Single-use reagents that are used on the automated system are stable on the Hamilton Microlab® STARlet deck for up to 4 hours prior to the start of the run.

Freeze-Thaw Stability.

Exact Sciences conducted a study to evaluate the stability of the *QuARTS* assay reagents when subjected to repeated freeze/thaw events. The *QuARTS* assay reagents tested included only those assay components normally stored frozen (-25 to -15°C):

1) Oligo Mix A, Methylation;
2) Oligo Mix B, Mutation;
3) Enzyme Mix;
4) DNA Calibrator 1 High Methylation;
6) DNA Calibrator 2 Low Methylation;
7) DNA Calibrator 3 High Mutation; and
8) DNA Calibrator 4, Low Mutation.

Materials from one lot of each assay component were subjected to 0, 2, 4, and 6 freeze-thaw cycles. Each component was then tested in the *Cologuard* molecular assay component using the *Cologuard* DNA Controls (i.e., DNA Control 1, High Positive and DNA Control 2, Low Positive), which did not undergo freeze-thaw cycling. The study tested 16 replicates for each component and each freeze-thaw cycle. Calibrators used during testing to assess assay validity and to generate curves for sample concentration assessment were not subjected to freeze-thaw cycling. Log strands for each marker were compared to those for samples where the reagents did not undergo freeze thaw cycling.

All log strand results for all samples were statistically equivalent to those that did not undergo freeze thaw cycling, thereby demonstrating that the *Cologuard QuARTS* assay reagents are stable for six freeze thaw events.

Real-Time Stability.

Exact Sciences is conducting an on-going study for real-time stability of *Cologuard*, evaluating the functional performance of three reagent lots over a period of 41 weeks. Each lot is comprised of unique batches of reagents, which will be tested at various time points over 41 weeks.

Samples that will be used to evaluate hemoglobin assay reagent stability consist of negative stool matrix spiked with whole blood to create samples with a low and high hemoglobin concentration. Samples for evaluation of molecular assay reagent stability consist of negative stool matrix spiked with oligonucleotides that contain the marker sequences. Oligonucleotides for *NDRG4, BMP3, BTACT, KRAS1, KRAS2,* and *ACT* will be spiked into the negative stool samples to create samples with a low and high level of sDNA samples. At each time point, seven replicates of samples and controls will be tested.

X. SUMMARY OF PRIMARY CLINICAL STUDY

A. Introduction and Background

The pivotal study ("Multi-Target Colorectal Cancer Screening Test for the Detection of Colorectal Advanced Adenomatous Polyps and Cancer: DeeP-C Study") demonstrated the safety and effectiveness of *Cologuard* as a screening test for the detection of markers associated with the presence of CRC and AA. In the pivotal trial, *Cologuard* demonstrated 92.3% sensitivity for CRC and 86.6% specificity, using colonoscopy with histopathological confirmation when required as the reference method. The study further compared CRC detection by *Cologuard* to that of a commercially available FIT (OC FIT-CHEK, Polymedco, Inc.) ("FIT"), demonstrating superiority (92.3% sensitivity for *Cologuard* compared with 73.8% sensitivity for FIT, $p=0.001$). Further *Cologuard* successfully demonstrated superiority to FIT with respect to advanced adenoma (AA) detection (42.4% sensitivity for *Cologuard*, compared with 23.8% sensitivity for FIT, $p<0.001$).

An overview of the study design and results is provided below.

B. Study Design

The *Cologuard* pivotal study was a prospective, multi-centered trial that began enrollment of study participants on June 30, 2011. A total of 12,776 patients were enrolled from 90 sites in the U.S. and Canada, including both colonoscopy centers and primary care sites, with study participation concluding on February 4, 2013. Subjects were provided with a collection kit, which they used to collect stool samples for *Cologuard* and FIT testing. Subjects subsequently underwent colonoscopy within 90 days of study enrollment.

The stool samples for analysis with *Cologuard* were sent to a central biorepository for batch testing at one of three laboratories while the stool samples for the FIT were sent to a single laboratory for testing. Samples tested with *Cologuard* were assayed by laboratory technicians blinded to the results of colonoscopy and the FIT results. Results from *Cologuard* and the FIT test were compared to the results of an optical colonoscopic examination, and histopathologic diagnosis of all significant lesions discovered during the colonoscopy and either biopsied or removed.

Colonoscopy findings were recorded per site specific standard of practice. Subjects with no findings were categorized as negative by colonoscopy. Histopathological results from biopsied tissue or excised lesions were categorized based on the most clinically significant lesion present (i.e. the index lesion) by a central pathologist according to the pre-specified standards outlined in **Table 8**

Table 8: Histopathological category definitions

Category	Findings
1	CRC, all stages (I-IV)
2	Advance adenoma, including the following subcategories: 2.1 – Adenoma with carcinoma *in situ*/high grade dyplasia, any size 2.2 – Adenoma, villous growth pattern ($\geq$25%), any size 2.3 – Adenoma $\geq$ 1.0 cm in size, or 2.4 – Serrated lesion, $\geq$ 1.0 cm in size
3	1 or 2 adenoma (s), >5 mm in size, or < 10 mm size, non-advanced
4	$\geq$ 3 adenomas, <10mm, non-advanced
5	1 or 2 adenoma(s), ≤5 mm in size, non-advanced
6	Negative – No neoplastic findings 6.1 – negative upon histopathological review 6.2 – no findings on colonoscopy, no histopathological review

C. Clinical Endpoints

The primary endpoint was the *Cologuard* sensitivity for CRC and specificity, using colonoscopy with histopathology (when required) as the reference method. The primary analysis required that the lower bound of the 95% one-sided confidence interval for the sensitivity of *Cologuard* for CRC exceed 65%. The specificity analysis required that the lower bound of the one-sided 95% confidence interval exceed 85%.

With respect to the secondary endpoints, *Cologuard* was compared to FIT using a non-inferiority test for CRC sensitivity and using a superiority test for advanced adenoma (AA) sensitivity. In order for *Cologuard* to be deemed non-inferior to FIT, the one-sided 95% confidence interval lower bound for the *Cologuard* – FIT difference in percentages with a positive test among subjects with CRC was required to exceed -5%. Establishing superiority required a one-sided p-value <0.025 (exact McNemar's comparison test).

D. Inclusion and Exclusion Criteria

Subjects eligible for enrollment in the study were of both genders between the ages of 50 and 84 years (inclusive), who were at average risk for development of colorectal cancer and asymptomatic for gastrointestinal symptoms warranting diagnostic colonoscopy. In addition, subject enrollment was age-weighted toward a slightly older population to increase the point prevalence of colorectal cancer in this study. An effort was made to enroll the majority of subjects of age 65-84; 64% of subjects in the actual study population were of age 65-84.

E. Accountability of PMA Cohort

The study enrolled a total of 12,766 subjects at 90 sites, including both primary care point-of-referral (POR) sites and colonoscopy centers. A total of 2,753 subjects were excluded from the primary analysis population due to unusable data (e.g., no colonoscopy). A total of 10,023 subjects were included in the primary analysis population. This population included 65 subjects with CRC. Analysis was conducted to rule out bias associated with the subjects excluded from the analysis population.

F. Study Population and Baseline Demographics

The baseline demographic characteristics for the Primary Effectiveness Population are presented in **Table 9** below. As shown in the table, the average age of subjects was 64.2 years old, and there was a slightly higher percentage of female subjects (5,378/10,023, 53.7%) as compared with male subjects (4,645 /10,023, 46.3%). The majority of subjects were White (8,422/10,017, 84.1%), although 10.7% of the population were Black or African American subjects (1,071/10,017). Nearly 10% of subjects were Hispanic or Latino (991/10,019, 9.9%). Average BMI was 28.83 and the majority of subjects never smoked (5,531 /10,019, 55.2%). It should be noted that two 49-year-old subjects and one 44-year-old subject were included in the study, which is inconsistent with the intended user population. Each of these subjects was a true negative and their inclusion did not notably impact data analyses.

Subjects that were enrolled at POR sites were similar to those enrolled at non-POR sites and to the population as a whole.

Table 9: Baseline Demographics – Primary Effectiveness Subjects

Parameter Statistic	All Enrolled (N=10023)	Specificity Subset (2-6) (N=9958)	Specificity Subset (3-6) (N=9198)	CRC Subset (N=65)	AA Subset (N=760)	FIT Secondary Effectiveness (N=65)
Age (years) at Screening						
n	10023	9958	9198	65	760	65
Mean (SD)	64.2 (8.42)	64.1 (8.41)	64.0 (8.44)	70.2 (7.92)	65.4 (7.93)	70.2 (7.92)
Median	66	66	66	70	66	70
Min, Max	44, 84	44, 84	44, 84	50, 84	50, 84	50, 84
Gender, n (%)						
Male	4645 (46.3)	4611 (46.3)	4161 (45.2)	34 (52.3)	450 (59.2)	34 (52.3)
Female	5378 (53.7)	5347 (53.7)	5037 (54.8)	31 (47.7)	310 (40.8)	31 (47.7)
Race, n (%)						
White	8422 (84.1)	8367 (84.1)	7726 (84.0)	55 (84.6)	641 (84.5)	55 (84.6)
Black or African American	1071 (10.7)	1063 (10.7)	978 (10.6)	8 (12.3)	85 (11.2)	8 (12.3)
Asian	259 (2.6)	258 (2.6)	245 (2.7)	1 (1.5)	13 (1.7)	1 (1.5)
American Indian or Alaska Native	36 (0.4)	36 (0.4)	32 (0.3)	0 (0.0)	4 (0.5)	0 (0.0)
Native Hawaiian or Other Pacific Islander	23 (0.2)	23 (0.2)	23 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Other	206 (2.1)	205 (2.1)	189 (2.1)	1 (1.5)	16 (2.1)	1 (1.5)
Missing	6	6	5	0	1	0
Ethnicity, n (%)						
Hispanic or Latino	991 (9.9)	982 (9.9)	923 (10.0)	9 (13.8)	59 (7.8)	9 (13.8)
Not Hispanic or Latino	9028 (90.1)	8972 (90.1)	8272 (90.0)	56 (86.2)	700 (92.2)	56 (86.2)
Missing	4	4	3	0	1	0
BMI (kg/m2) at Baseline						
n	10015	9950	9190	65	760	65
Mean (SD)	28.83 (5.836)	28.84 (5.841)	28.77 (5.817)	27.55 (4.861)	29.67 (6.068)	27.55 (4.861)
Median	28.0	28.0	27.9	26.8	29.0	26.8
Min, Max	13.3, 68.2	13.3, 68.2	13.3, 68.2	19.3, 42.4	16.3, 59.9	19.3, 42.4
Smoking History, n (%)						
Never Smoked	5531 (55.2)	5498 (55.2)	5157 (56.1)	33 (50.8)	341 (44.9)	33 (50.8)
Former Smoker	3589 (35.8)	3564 (35.8)	3279 (35.6)	25 (38.5)	285 (37.5)	25 (38.5)
Current Smoker	903 (9.0)	896 (9.0)	762 (8.3)	7 (10.8)	134 (17.6)	7 (10.8)
If Former or Current Smoker, Daily Use, n (%)						
<1/2 Pack Per Day	2162 (48.3)	2154 (48.4)	1970 (48.9)	8 (25.0)	184 (44.0)	8 (25.0)
1 Pack Per Day	1585 (35.4)	1569 (35.3)	1418 (35.2)	16 (50.0)	151 (36.1)	16 (50.0)
>1 Pack Per Day	732 (16.3)	724 (16.3)	641 (15.9)	8 (25.0)	83 (19.9)	8 (25.0)
Missing	13	13	12	0	1	0
If Former or Current Smoker, # Years Smoking						
n	4480	4448	4029	32	419	32
Mean (SD)	21.82 (14.733)	21.77 (14.732)	21.13 (14.450)	28.47 (13.488)	27.93 (15.959)	28.47 (13.488)
Median	20.0	20.0	20.0	29.0	30.0	29.0
Min, Max	0.0, 70.0	0.0, 70.0	0.0, 70.0	1.0, 60.0	1.0, 65.0	1.0, 60.0

G. Primary Effectiveness Evaluations (Sensitivity/Specificity)

Results from the DeeP-C study demonstrated that *Cologuard* successfully met the primary endpoint of the study, establishing a clinically meaningful sensitivity and specificity for CRC. Specifically, as shown in the table below, sensitivity of *Cologuard* for CRC was 92.3% (60/65) with a one-sided 95% confidence interval lower bound of 84.5

Table 10: Overall Sensitivity for CRC – Primary Effectiveness Subjects

	Valid *Cologuard* (N=65) Positive Result
Case Category, n/N (%)	
1: CRC Stages 1-4	60/65 (92.3%)
Sensitivity Based on Category 1: Primary (one-sided 95% CI lower bound)	92.3% (>84.5%)
Sensitivity Based on Category 1: Supportive (one-sided 97.5% CI lower bound)	92.3% (>83.0%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculated using an exact one-sided binomial test.

In addition, *Cologuard* successfully demonstrated a clinically meaningful specificity according to the protocol-specified criteria. As shown in **Table 11** below, the specificity of *Cologuard* was 86.6%, with a one-sided 95% confidence interval lower bound of 86.0%. Thus, the study was a success with respect to specificity.

Table 11: Overall Specificity – Primary Effectiveness Subjects

	Valid *Cologuard* (N=9198) Negative Result
Case Category, n/N (%)	
3: 1-2 Adenomas 5-<10 mm	607/749 (81.0%)
4: >=3 Adenomas <10 mm, Non-advanced	302/419 (72.1%)
5: 1-2 Adenomas <5 mm, Non-advanced	1496/1735 (86.2%)
6.1: Negative upon histopathological review	1543/1821 (84.7%)
6.2: No findings on colonoscopy, no histopathological review	4019/4474 (89.8%)
Specificity Based on Categories 3-6: Primary (one-sided 95% lower bound)	86.6% (>86.0%)
Specificity Based on Categories 3-6: Supportive (one-sided 97. 5% lower bound)	86.6% (>85.9%)

[1] Percentages based on valid test results within a category.
[2] Lower bounds calculates using an exact one-sided binomial test.
[3] As noted above, one 44-year-old and two 49-year-old true negative subjects were included in the analysis population, although they would not be included in the intended user population.

H. Secondary Effectiveness Evaluations

Cologuard was compared to FIT using a non-inferiority test for CRC sensitivity and using a superiority test for advanced ademoma (AA) sensitivity.

The primary and secondary endpoint analyses demonstrate that *Cologuard* is highly sensitive for CRC and has a significant sensitivity advantage over the FIT. As shown in **Table 12**, sensitivity of *Cologuard* for CRC was 92.3% (60/65), compared with 73.8% (48/65) for FIT. In order for *Cologuard* to be deemed non-inferior to FIT, the one-sided 95% confidence interval lower bound for the *Cologuard* – FIT difference in percentages with a positive test among subjects with CRC was required to exceed -5%. The lower bound of the one-sided confidence interval for the Cologuard – FIT difference was 8%, substantially exceeding the protocol-specified non-inferiority threshold. As shown in the 2x2 table below, *Cologuard* correctly captured 60 of the 65 total CRC cases identified by colonoscopy (92.3%). Meanwhile, FIT captured only 48 of the 65 CRC cases identified by colonoscopy (73.8%). Notably, FIT identified only a single cancer that was not identified by *Cologuard*. *Cologuard*, meanwhile, identified 13 cancers that were missed by FIT.

As the non-inferiority analysis was satisfied, the protocol allowed for a superiority analysis comparing *Cologuard* to FIT for CRC sensitivity. In this analysis, an exact McNemar's comparison test was performed; a one-sided p-value <0.025 was required to achieve superiority. *Cologuard* demonstrated superiority over FIT with respect to sensitivity for CRC as the one-sided p-value (p=0.0018) was well below the p <0.025 threshold for superiority.

Table 12: Overall Sensitivity: CRC Subset (Category 1) - Secondary Effectiveness Subjects

	Valid *Cologuard* (N=65) Positive Result	**Valid FIT (N=65) Positive Result**
Case Category, n/N (%)		
1: CRC Stages 1-4	60/65 (92.3%)	48/65 (73.8%)
Sensitivity Based on Category 1: Primary (one-sided 95% lower bound)	92.3% (>84.5%)	73.8% (>63.4%)
Specificity Based on Categories 3-6: Supportive (one-sided 97. 5% lower bound)	92.3% (>83.0%)	73.8% (>61.5%)

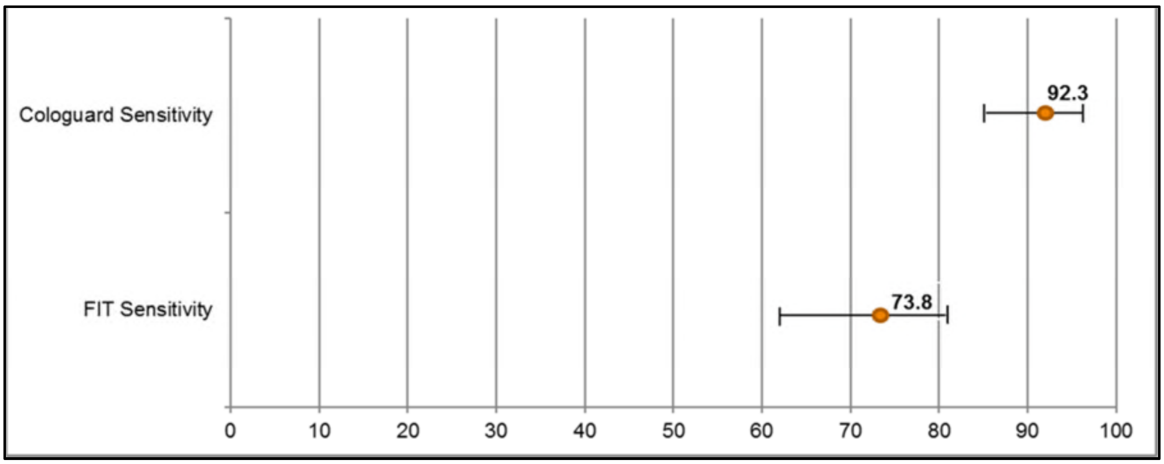
[1] Percentages based on valid test results within a category.
[2] Lower bounds calculated using an exact one-sided binomial test.

Table 13: Sensitivity Non-Inferiority and Superiority Test – CRC Subset (Category 1)

	***Cologuard* Outcome**	**FIT Outcome**			
		Negative	**Positive**	**Totals**	**McNemar test p-value**
Category 1	Negative, n (%)	4 (80.0)	1 (20.0)	5	0.0018
	Positive, n (%)	13 (21.7)	47 (78.3)	60	
	Totals	17	48	65	

[1] p-value is from a McNemar paired comparison test of the discordant pairs.
[2] One-sided 5% lower bound on the discordant pair difference for Category 1 = 0.080 > -0.050.
[3] One-sided 2.5% lower bound on the discordant pair difference for Category 1 = 0.060 > -0.025.

Figure 1: CRC Sensitivity



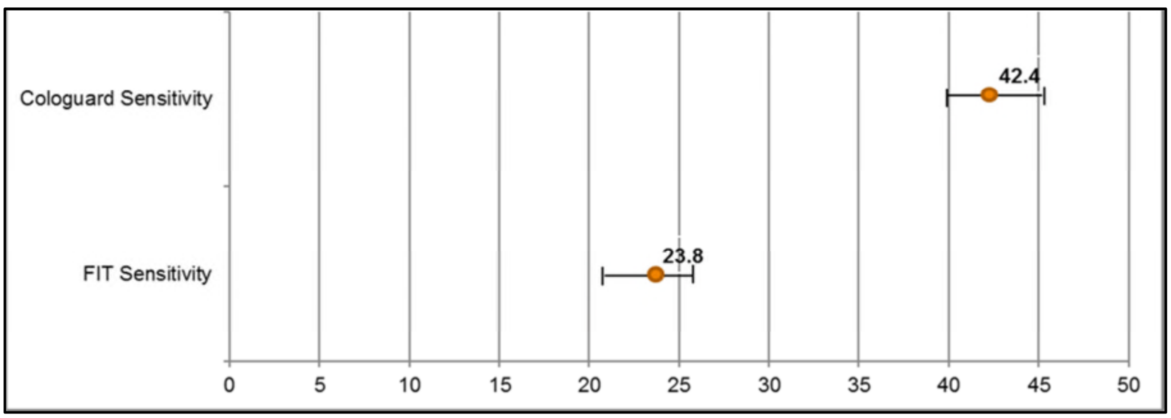
The secondary endpoint analyses also evaluated *Cologuard's* sensitivity for histopathologically-confirmed AAs, compared to FIT, using a superiority test. As shown in **Table 14** below, overall sensitivity for AA was 42.4% for *Cologuard* compared with 23.8% for FIT. In order to establish superiority for AA sensitivity, the protocol required a one-sided p-value of less than 0.025. *Cologuard* successfully demonstrated superiority over FIT with respect to sensitivity for AA as the one-sided p-value (p<0.0001) was well below the p <0.025 threshold for superiority. FIT identified only 29 AA cases that were not captured by *Cologuard*, while *Cologuard* identified 170 AA cases that were not positive on the FIT test.

Table 14: Sensitivity Superiority Test – AA Subset (Category 2)

		FIT Outcome			
	***Cologuard* Outcome**	**Negative**	**Positive**	**Totals**	**McNemar test p-value**
Category 2	Negative, n (%)	407 (93.3)	29 (6.7)	436	<0.0001
	Positive, n (%)	170 (53.0)	151 (47.0)	321	
	Totals	577	180	757	

[1] p-value is from a McNemar paired comparison test of the discordant pairs.
[2] One-sided 5% lower bound on the discordant pair difference for Category 2 = 0.147 > -0.050.
[3] One-sided 2.5% lower bound on the discordant pair difference for Category 2 = 0.140 > -0.025.

Figure 2: AA Sensitivity



The combined sensitivity for CRC and AA subjects was also analyzed and is provided in **Table 15** below. As shown in the table, *Cologuard* sensitivity is 46.3% while FIT sensitivity is 27.7%. Even under this analysis, *Cologuard* maintained a 15-20% absolute advantage in sensitivity over FIT.

Table 15: Sensitivity for Advanced Neoplasia (CRC + AA)

	***Cologuard* N=822**	**PolyMedco FIT N=822**
	Sensitivity	Sensitivity
Category 1 Only	92.3% (60/65)	73.8% (48/65)
Categories 1-2	46.4% (381/822)	27.7% (228/822)

Numerically greater sensitivity for *Cologuard* compared to FIT was observed across all sub-categories of AA. For example, sensitivity for adenoma with carcinoma in situ or high grade dysplasia (Category 2.1) was 69.2% for *Cologuard*, compared to 46.2% for FIT. Importantly, *Cologuard* identified 43.0% of serrated lesions, which historically have been difficult to capture with FIT, due to the fact that these lesions do not bleed. FIT sensitivity for these lesions was only 5.1%.

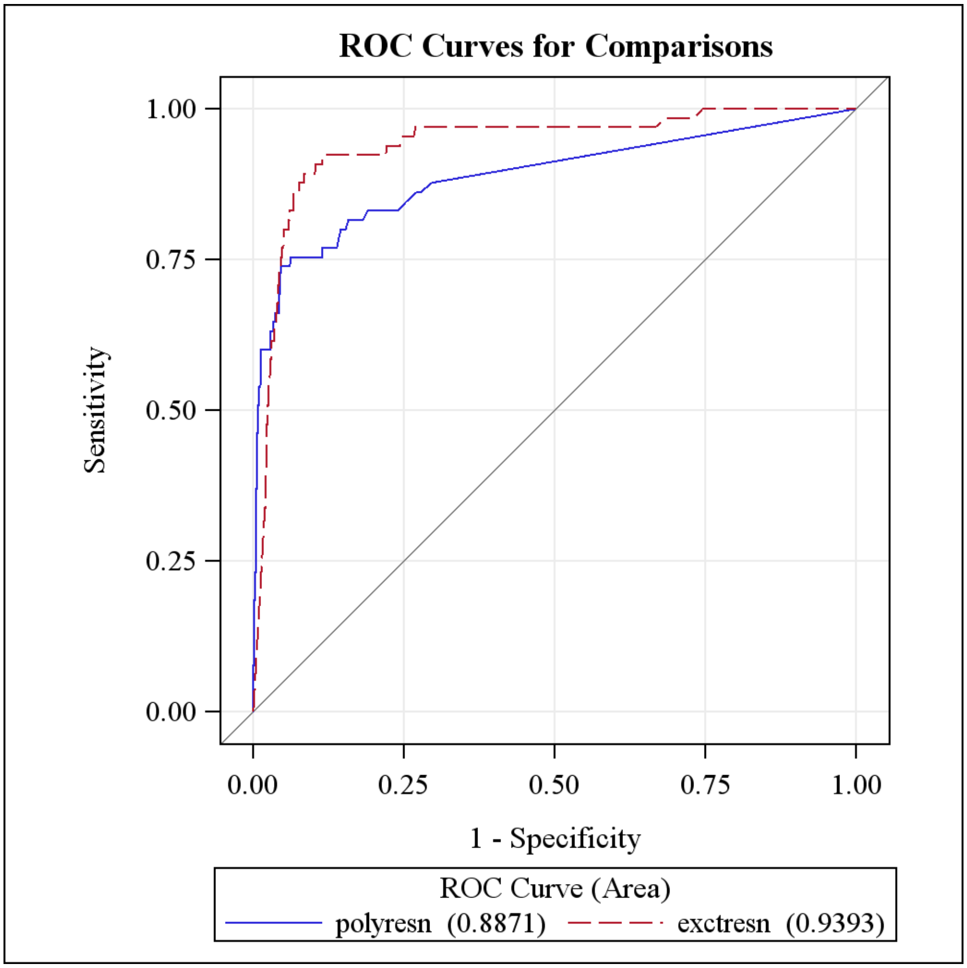
With respect to specificity, the protocol did not plan for a formal comparison to FIT as the two tests are designed to have different specificities. However, the company compared the number of true negatives captured by *Cologuard* out of those identified by colonoscopy (7,936/9,167, 86.6%), to those captured by FIT, as shown in the 2x2 table below. FIT captured more true negatives, (8,695/9,167 94.9%), but the FIT test by design has a higher specificity, and consequently, significantly lower sensitivity than *Cologuard.*

Table 16: Specificity – Specificity Subset (Categories 3-6)

		FIT Outcome		
	***Cologuard* Outcome**	**Negative**	**Positive**	**Totals**
Categories 3-6	**Negative, n (%)**	7787 (98.1)%	149 (1.9)%	7936
	Positive, n (%)	908 (73.8)%	323 (26.2)%	1231
	Totals	8695	472	9167

In addition, a Receiver Operating Characteristic curve ("ROC curve") was generated for the sensitivity and specificity of *Cologuard*, compared to FIT, for the primary specificity analysis in which AA cases were considered true positives and excluded from the analysis (Categories 3-6). The results of this analysis, shown in **Figure 3** below, further demonstrate the robust performance of *Cologuard*. The area under the curve (AUC) for *Cologuard* indicates that a randomly chosen CRC patient is 93.9% more likely to have a higher test value than a patient in Categories 3-4, while that percentage is 88.7% for FIT. The two sided p-value for this comparison was statistically significant (p=0.0372).

Figure 3: CRC Sensitivity using Categories 3-6 for Specificity Cologuard (exctresn) vs. FIT (polyresn)



I. Additional Effectiveness Analyses

In addition to the sensitivity and specificity for CRC and AA, the positive and negative likelihood ratios for *Cologuard* were calculated from the study data. Results demonstrated a positive likelihood ratio of 6.9 for CRC, indicating that a person with CRC would be 6.9 times more likely to have a positive *Cologuard* results than someone without CRC. The negative likelihood ratio for CRC was 0.089, indicating that someone without CRC is approximately 11 times (1/0.089) more likely to test negative on *Cologuard* compared to someone with CRC. Results were similarly robust for AA.

Table 17: Positive and Negative Likelihood Ratios

	Category 1 (CRC) vs Categories 3-6	**Category 2 (AA) vs Categories 3-6**
Positive Likelihood Ratio (PLR)		
Sensitivity	92.3	42.4
1-Specificity	13.4	13.4
PLR	6.897	3.166
95% Confidence Interval	(6.320, 7.527)	(2.871, 3.491)
Negative Likelihood Ratio (NLR)		
1-Sensitivity	7.7	57.6
Specificity	86.6	86.6
NLR	0.089	0.665
95% Confidence Interval	(0.038, 0.206)	(0.626, 0.708)

Analysis was also performed to calculate the positive and negative predictive values ("PPV" and "NPV") for *Cologuard*. As with any CRC screening test, the PPV is impacted by the very low prevalence of CRC in the general population. The PPV was calculated to be 3.72% (60/1613) for CRC and 19.86% (322/1613) for AA. Meanwhile, the NPV was 94.73%.

Table 18: Positive Predictive Value – Primary Effectiveness Subjects

Cologuard	**Category 1 (CRC)**	**Category 2 (AA)**	**Categories 3-6**
Negative	0.06, 0.02-0.14 (5/8410)	5.21, 4.74-5.71 (438/8410)	94.73, 94.23-95.20 (7967/8410)
Positive	3.72, 2.85- 4.76 (60/1613)	19.96, 18.0-22.0 (322/1613)	76.32, 74.16-78.37 (1231/1613)

*2-Sided 95% CIs

Sub-Group Analyses

The DeeP-C study results were also analyzed according to various demographic characteristics, as well as lesion size and location.

Results by Gender

Sensitivity of *Cologuard* was higher for males than for females, both for CRC and AA. As shown in **Table 19** below, *Cologuard* sensitivity for CRC was 100.0% for males, compared with 83.9% for females. Sensitivity for AA was 44.7% for males, compared with 39.0% for females.

Table 19: *Cologuard* Sensitivity by Gender (Categories 1 and 2)

Subgroup	Category 1 (CRC)	Category 2 (AA)
Gender, n/N (%)		
Male	34/34 (100.0)	201/450 (44.7)
Female	26/31 (83.9)	121/310 (39.0)

[1] Sensitivity calculated as number of positives (CRC or AA) divided by subjects with CRC or AA, respectively.

Meanwhile, specificity of *Cologuard* was very similar for females as compared with males. As shown in **Table 20** below, specificity for CRC was 87.3% (4,398/5,037) for females, compared with 85.8% (3,569/4,161) for male subjects.

Table 20: *Cologuard* Specificity by Gender

Subgroup	Categories 3-6[1]
Gender, n/N (%)	
Male	3569/4161 (85.8)
Female	4398/5037 (87.3)

[1] Specificity calculated as number of negatives among subjects without CRC or AA.

Results by Race and Ethnicity

With respect to race, *Cologuard* sensitivity for CRC was higher among White subjects (53/55, 96.4%), than among Black or African-American subjects (5/8, 62.5%) and higher among the small number of Asian CRC cases (1/1, 100.0%). However, the results observed in Black or African-American subjects may well have been driven by the low overall number of cancer cases in that subpopulation. Notably, sensitivity among Hispanic or Latino subjects (8/9, 88.9%) was high, although again the sample size was small. As shown in **Table 21** below, sensitivity for AA was similar for White (271/641 42.3%) and Black/African-American (36/85, 42.4%) subjects. Sensitivity was also similar among Hispanic/Latino subjects (23/59, 39.0%). *Cologuard* sensitivity for AA was lower among Asian subjects (4/13, 30.8%) and very high for American Indian or Alaskan Natives (3/4, 75.0%), compared with other groups.

Table 21: *Cologuard* Sensitivity by Race and Ethnicity, CRC and AA Subsets (Categories 1 and 2)

Subgroup	Category 1 (CRC)	Category 2 (AA)
Race, n/N (%)		
White	53/55 (96.4)	271/641 (42.3)
Black or African American	5/8 (62.5)	36/85 (42.4)
Asian	1/1 (100.0)	4/13 (30.8)
American Indian or Alaska Native	0/0	3/4 (75.0)
Native Hawaiian or Other Pacific Islander	0/0	0/0
Other	1/1 (100.0)	7/16 (43.8)
Ethnicity, n/N (%)		
Hispanic or Latino	8/9 (88.9)	23/59 (39.0)
Not Hispanic or Latino	52/56 (92.9)	298/700 (42.6)

[1] Sensitivity calculated as number of positives (CRC or AA) divided by subjects with CRC or AA.

Cologuard specificity for CRC was high across all racial and ethnic groups, with rates > 85% for most groups. Specificity rates were highest for Asian and Native Hawaiian/Pacific Islander subjects and lowest for American Indian/Alaska Native subjects. Specificity was 93.5% (229/245) for Asian subjects, and 91.3% (21/23) for Native Hawaiian/Pacific Islander subjects. Specificity was also high (90.7% (837/923)) among Hispanic or Latino subjects. Specificity was similar for White (6,639/7,726, 85.9%) and Black/African-American (879/978, 89.9%) subjects in this analysis, and lowest for American Indian/Alaskan Native subjects (24/32, 75.0%), as shown in **Table 22** below.

Table 22: *Cologuard* Specificity by Race and Ethnicity – Primary Effectiveness Subjects

Subgroup	Categories 3-6
Race, n/N (%)	
White	6639/7726 (85.9)
Black or African American	879/978 (89.9)
Asian	229/245 (93.5)
American Indian or Alaska Native	24/32 (75.0)
Native Hawaiian or Other Pacific Islander	21/23 (91.3)
Other	171/189 (90.5)
Ethnicity, n/N (%)	
Hispanic or Latino	837/923 (90.7)
Not Hispanic or Latino	7127/8272 (86.2)

[1] Specificity calculated as number of negatives among subjects without CRC or AA.

Results by Age

Cologuard sensitivity for CRC was consistently high across all age groups, as shown in **Table 23** below. Sensitivity for patients 65 years of age and older ranged from 88.9% to 100.0%. Although sensitivity was 75% for subjects age 60-64, the number of CRC cases was particularly small in this age group (n = 4); only one CRC case was not detected by *Cologuard*. With respect to AA, sensitivity was similar across all age groups, with sensitivity as high as 46.8% for subjects between the ages of 70 and 79.

Table 23: *Cologuard* Sensitivity by Age

Subgroup	**Category 1 (CRC)**	**Category 2 (AA)**
Age, n/N (%)		
<60 years	7/7 (100.0)	65/171 (38.0)
60-64 years	3/4 (75.0)	24/57 (42.1)
65-69 years	19/20 (95.0)	125/301 (41.5)
70-74 years	16/18 (88.9)	72/154 (46.8)
75-79 years	6/6 (100.0)	29/62 (46.8)
>79 years	9/10 (90.0)	7/15 (46.7)

[1] Sensitivity calculated as number of positives (CRC or AA) divided by subjects with CRC or AA.
[2] Two 49-year-old subjects and one 44-year-old subject were included in the analysis population, although they would not be included in the intended use population.

Cologuard specificity for CRC was also high across all age groups. As shown in **Table 24** below, specificity was highest for younger subjects and lower for older subjects. Specificity was in the 80% range or above for most age groups, aside from subjects > 75 years old.

Specificity for AA was also similar across age groups, and like specificity for CRC, was highest for younger subjects and slightly lower for older subjects.

Table 24: *Cologuard* Specificity by Age

Subgroup	**Categories 3-6**
Age, n/N (%)	
<60 years	2491/2703 (92.2)
60-64 years	681/765 (89.0)
65-69 years	2871/3352 (85.7)
70-74 years	1292/1566 (82.5)
75-79 years	480/617 (77.8)
>79 years	152/195 (77.9)

[1] Specificity calculated as number of negatives among subjects without CRC or AA.
[2] Two 49-year-old subjects and one 44-year-old subject were included in the analysis population, although they would not be included in the intended use population.

Results by Lesion Size and Cancer Stage

Exact Sciences evaluated *Cologuard* results by lesion size, as well as cancer stage. Sensitivity of *Cologuard* decreased with lesion or lesion size, as would be expected for a stool-based DNA test of this type. The amount of DNA shed from cancerous or pre-cancerous tissue in the colon is generally expected to increase with increased mass or lesion size.

As shown in the table below, sensitivity was > 90% for most lesion sizes. Sensitivity for CRC was highest for subjects with CRCs ≥ 30 mm (32/34, 94.1%) and lowest for subjects with CRCs 5-9 mm in size (4/5, 80.0%). Sensitivity by cancer stage was generally high and was the highest for subjects with Stage II cancers (21/21, 100.0%) and Stage III cancers (9/10, 90%). Sensitivity of *Cologuard* for AA was also higher among subjects with AAs of larger sizes.

Table 25: *Cologuard* Sensitivity within Lesion Subgroups

Subgroup	**Category 1 (CRC)**	**Category 2 (AA)**
Largest Lesion Size, n/N (%)		
<5 mm	0/0	2/10 (20.0)
5-9 mm	4/5 (80.0)	18/56 (32.1)
10-19 mm	13/14 (92.9)	225/577 (39.0)
20-29 mm	11/12 (91.7)	51/79 (64.6)
>=30 mm	32/34 (94.1)	26/38 (68.4)
Stage, n/N (%)		
I	26/29 (89.7)	N/A
II	21/21 (100.0)	N/A
III	9/10 (90.0)	N/A
IV	3/4 (75.0)	N/A
Unknown*	1/1 (100.0)	N/A

[1] Sensitivity calculated as number of positives (CRC or AA) divided by subjects with CRC or AA.

Specificity of *Cologuard* by lesion size is shown in **Table 26** below. As shown in the table, specificity of *Cologuard* for CRC was 86.2% (1,847/2,142), for subjects with CRCs < 5 mm in size, and 79.7% (1,523/1,912) for subjects with CRCs 5-9 mm in size.

Table 26: *Cologuard* Specificity by Lesion Size – Primary Effectiveness Subjects

Subgroup	**Categories 3-6**
Largest Lesion Size, n/N (%)	
<5 mm	1847/2142 (86.2)
5-9 mm	1523/1912 (79.7)
10-19 mm	0/0
20-29 mm	0/0
>=30 mm	0/0

[1] Specificity calculated as number of negatives among subjects without CRC or AA.

Results by Lesion Location

Cologuard results also were assessed by lesion location. As shown in **Table 27** below, sensitivity of *Cologuard* for CRC was 90% or greater, regardless of lesion location. Sensitivity of *Cologuard* for AA was greatest among subjects with distal AAs (133/238, 55.9%).

Table 27: *Cologuard* Sensitivity by Lesion Location

Subgroup	Category 1 (CRC)	Category 2 (AA)
Lesion Location, n/N (%)		
Proximal	27/30 (90.0)	143/433 (33.0)
Distal	22/24 (91.7)	133/238 (55.9)
Rectal	11/11 (100.0)	45/88 (51.1)

[1] Sensitivity calculated as number of positives (CRC or AA) divided by subjects with CRC or AA.

Specificity of *Cologuard* for CRC was high, regardless of lesion location. Specificity of *Cologuard* was 83.4% for subjects with proximal CRCs, 82.1% for subjects with distal CRCs, and 84.5% for subjects with rectal CRCs.

Table 28: *Cologuard* Specificity by Lesion Location – Primary Effectiveness Subjects

Subgroup	Categories 3-6
Lesion Location, n/N (%)	
Proximal	1723/2066 (83.4)
Distal	1131/1377 (82.1)
Rectal	517/612 (84.5)

[1] Specificity calculated as number of negatives among subjects without CRC or AA.

Safety Analyses

With respect to safety, due to the design of the study and the nature of the stool collection process, AEs caused by or related to the stool collection procedure were not expected. As a result, events associated with potential errors in use of the collection kit and any product complaints were captured in the safety analyses. There were no cases in which the study investigator believed the product contributed to a serious adverse event, and only 4 adverse events were reported. Events included a broken fingernail, cut finger, leg pain related to a fall during stool collection and sprained hand. None of the AEs experienced in the study were deemed “serious”, all were categorized as “mild” events. None of the events led to the subject discontinuing the study.

Additionally, one subject died of unrelated causes prior to undergoing colonoscopy. The subject met all eligibility criteria and successfully collected a stool sample, but did not present for the subsequent colonoscopy.

XI. CONCLUSIONS DRAWN FROM NONCLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

Risks associated with the collection of the stool sample necessary for the *Cologuard* test were very minimal. During the pivotal clinical trial of 12,776 patients, only 4 mild adverse events were reported.

With respect to the *Cologuard* test itself, the primary risk relates to a false assay result (i.e., a false positive or a false negative result). All positive test results should lead to a colonoscopy. Adverse events commonly associated with colonoscopy include abdominal discomfort and bowel irregularity post-procedure. Rare adverse events associated with colonoscopy include bleeding, intestinal perforation, and adverse reaction to the sedation resulting in respiratory and/or cardiac events, stroke and death. In the instance of a false negative result on *Cologuard*, there is a possibility that a case of CRC or AA could go undetected.

B. Effectiveness Conclusions

Data from the analytical studies demonstrated acceptable analytical sensitivity, analytical specificity and precision and reproducibility of *Cologuard*.

Similarly, data collected during the pivotal clinical trial demonstrated that *Cologuard* is safe and effective as a screening test for the detection of markers associated with the presence of CRC and AA. The study established *Cologuard* sensitivity for CRC of 92.3% and a specificity of 86.6%. The lower bounds of the one-sided 95% confidence intervals for these results exceeded the thresholds set in the study protocol. As such, *Cologuard* clearly satisfied the primary endpoint for the study.

In addition, the study successfully demonstrated superiority of *Cologuard* to FIT for detection of CRC ($p=0.0018$) and AA ($p<0.0001$). *Cologuard* demonstrated significant incremental benefit over FIT, identifying 13 CRC cases that were not identified by FIT. Meanwhile, in only 1 case did FIT identify a CRC case that was not identified by *Cologuard*. Overall, *Cologuard* yielded a 20.0% incremental benefit over FIT for CRC detection. Similarly, for AA detection, *Cologuard* successfully identified 178 AA cases that were not identified by FIT. Meanwhile, FIT only identified 29 AA cases that were not identified by *Cologuard.* Overall, *Cologuard* had a 22.5% incremental benefit for AA detection. Additionally, *Cologuard* sensitivity for adenoma with carcinoma *in situ*/high grade dysplasia was 69.2%, compared to only 46.2% for FIT. *Cologuard* also identified a notable percentage of serrated lesions (42.4%), which historically have been difficult to capture with FIT, due to the fact that these lesions do not bleed. FIT sensitivity for serrated was only 5.1%. Finally, *Cologuard* sensitivity for CRC was demonstrated across a variety of age groups, racial/ethnic groups, and in both men and women.

In conclusion, the pivotal study was a success, demonstrating that *Cologuard* met and exceeded the primary endpoint of the study. Additionally, *Cologuard* met and exceeded the secondary endpoints of the study, demonstrating non-inferiority and superiority to FIT. *Cologuard* was highly sensitive and specific for CRC and provides significant incremental value over currently available non-invasive screening tests for CRC.

C. Benefit-Risk Conclusions

Colorectal cancer (CRC) is the second leading cause of death from cancers affecting both men and women in the United States. A 2012 report from the American Cancer Society (ACS) estimated that 1 in 19 males and 1 in 20 females will develop CRC during his or her lifetime.[3] The National Cancer Institute further estimates that there will be 142,820 new cases of CRC and 50,830 deaths from this disease in the United States in 2013.[4]

Current guidelines for CRC screening in the average-risk population recommend regular screening of both men and women starting at age 50, as the incidences of both CRC and premalignant lesions increase sharply after this age.[5] When diagnosed at an early stage, the relative 5-year survival rate for colorectal cancer is approximately 90%. However, once the cancer has spread to nearby organs or lymph nodes, the 5-year relative survival rate is approximately 11%.[6] As such, early detection through screening provides a survival benefit.

The probable benefits of the device are based on data collected in the clinical study ("DeeP-C") conducted to support PMA approval as described above. The clinical benefit of *Cologuard* was demonstrated in an analysis of efficacy and safety data obtained from patients who are typical candidates for colorectal cancer screening, adults of either sex, 50 years or older, who were at average risk for colorectal cancer (DeepP-C Study). Based on these data, *Cologuard* provides a safe and effective additional adjunctive screening tool for detection of CRC.

When used for screening, a positive result should be followed by colonoscopy for diagnosis. The risks associated with the device are similar to other *in vitro* diagnostic assays and are associated with risks resulting from false results. A false positive result could result in an additional invasive screening procedure, such as colonoscopy, and thus expose patients to the attendant risks associated with such a procedure. A false negative result with *Cologuard* could potentially delay colonoscopy and a potentially delayed diagnosis of disease. The clinical data in this application demonstrated that the *Cologuard* was highly sensitive and specific for CRC and provides significant incremental value over currently available non-invasive screening tests for CRC. Furthermore, data from the analytical studies demonstrated acceptable analytical performance of the test. Given these data, the benefits to patients tested with *Cologuard* outweigh potential risks when used in accordance with the device labeling.

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XII. PANEL MEETING RECOMMENDATIONS AND FDA'S POST-APPROVAL ACTION

[TO BE COMPLETED BY FDA]

XIII. CDRH DECISION

[TO BE COMPLETED BY FDA]

XIV. APPROVAL SPECIFICATIONS

[TO BE COMPLETED BY FDA]

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